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Effects of Individuals and Behaviors on Acoustic Features of Ultrasonic Vocalizations in Rats

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ABSTRACT : The goal of this study was to investigate how spectrographic features of ultrasonic vocalizations (USVs) in rats vary among individuals and behaviors. Eighteen pairs of rats were allocated to individual pair cages. Each pair's behaviors and vocalizations were recorded during the 900s a known cage-mate was returning to the cage. The effects of individuals, behaviors, and the interaction between individuals and behaviors (individuals×behaviors) were tested on the duration and peak frequencies. There was difference in the duration and peak frequency: i) among individuals ($p<0.0001$ and $p<0.0001$, respectively); ii) among behaviors ($p = 0.0667$ and $p< 0.0001$, respectively); iii) among individuals×behaviors ($p<0.0001$ and $p<0.0001$, respectively). The frequency of ultrasonic vocalizations changed with a frequency ranging from 40 to 71 kHz which were emitted by individuals, whereas the frequency of ultrasonic vocalizations changed with a frequency ranging from 60 to 70 kHz which were emitted by behaviors. The peak frequency of call on 'contact' behavior was lower than that of call on other behaviors, but call duration of call on 'contact' was longer than on other behaviors. Especially, 40 kHz calls were found on 'contact' and 'other' behaviors. We suggest that ultrasonic vocalizations need to be subdivided and the effects of individuals and behaviors must be considered to assess emotional state of rats because these may influence the features of ultrasonic vocalizations. (**Key Words :** Ultrasonic Vocalization, Acoustic Feature, Rat, Individual, Behavior)

INTRODUCTION

Ultrasonic vocalizations (USVs) of rats have received considerable attention in recent literature. Vocalizations produced by adult rats are typically categorized as 22 kHz and 50 kHz calls which are negatively and positively associated with emotion, respectively (Knutson et al., 2002; Burman et al., 2006; Burgdorf and Panksepp, 2006; Burgdorf et al., 2007; Wohr et al., 2008). Previous studies showed that 22 kHz calls are associated with pain (van der Poel et al., 1989; Kaltwasser, 1990; Jourdan et al., 1995; Calvino et al., 1996).

Rats vocalize under a range of conditions including sexual interaction (Paredes and Alonso, 1997) and aggressive encounter (Sales, 1972). The 22 kHz calls are associated with defensive postures (Portavella et al., 1993), and produced when rats are exposed to a predator and under aggressive encounters (Sales and Pye, 1974; Blanchard et al., 1991). The 50 kHz calls are apparently produced in anticipation of social contact (Brudzynski and Pniak, 2002). However, much of the previous research on this topic has focused on just a few call features, and more studies need to be conducted to describe the behaviors related to the vocalized calls in various situations.

Few studies have described the spectrographic features of these calls (Brudzynski et al., 1999; Kehoe et al., 2001; Brudzynski and Pniak, 2002) and none to date has shown how these call features vary in various individuals and behaviors. Therefore, the aim of this study was to describe how spectrographic features of ultrasonic vocalizations of the rat vary in various individuals and behaviors.

MATERIALS AND METHODS

Subject

Eighteen pairs of female rats (Wistar; 400-500 g) were obtained from the UBC (University of British Columbia) Animal Care Center Rodent Breeding Unit as surplus supply stock. Animal room temperature was kept at 21°C and the light was turned on and off every 12 hours in turn. Rats could freely access food (Lab Diet 5001, PMI Nutrition International, Richmond, USA) and tap water. All testing was conducted during the period the light was on.

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Table 1. The mutually exclusive categories used for behavior observation

Behavior	Description
Contact	Nose to nose contacting, which included touching each other
Move	Quadrupedal ambulatory movement, which included partial or full extension bipedal
Other	Either licking or scratching in grooming bout, or not moving at all

Experimental procedure and analysis

Each of the 18 pairs was allocated to an individual cage (45.5 cm×24 cm×20.5 cm; 20 L polypropylene cage). Because rats are highly social animals, each experimental animal was housed with a companion to encourage USV production. Behaviors were recorded using a Panasonic WV-BP330 camera and AG-6720A VCR. Each pair was recorded continuously during the first 0-300 s and 600-900 s a known cage-mate was returning to the cage after an ovariectomy performed as part of another study. In that study, analgesics were administered one hour prior to surgery. Following treatment, rats were allowed to recover in an incubator for one hour. The recorded behavioral patterns are described in Table 1. Behaviors were scored for frequency and duration using the Observer (Noldus, Netherlands).

Rat vocalizations were collected with a 1/4" condenser microphone (Bruel and Kjaer, Type 4135, Denmark), connected to a preamplifier (Bruel and Kjaer, Type 2619, Denmark) and a measuring amplifier (Bruel and Kjaer, Type 2636, Denmark). The signal was recorded directly to a high-capacity hard disk at a rate of 250 kHz using a 330 kHz PCI-DAS1200/JR data acquisition card (Computerboards Inc.) and CBDisk 1.4 Software (Engineering Design, Belmont, MA, USA). High (above 100 kHz) and low frequency room noise were filtered out by a Krohn-Hite band pass filter. Sound analysis was done by SIGNAL 4.0 (Engineering Design, Belmont, MA, USA).

To divide into individuals, vocalizations could be detected using the Signal and then we confirmed the individual behavior using the Observer at that time. Vocalizations were subjected to spectrographic analysis to determine call duration and peak frequency (Niel and Weary, 2006). These parameters may be useful for comparing ultrasonic calls as they varied considerably in call shape (Callahan et al., 1996; Brudzynski et al., 1999), frequency, and duration (Sales, 1979).

Statistical analysis

In order to compare correctly, some pairs (emitting 22 kHz call continuously or call nothing; 13 pairs) were excluded from all data analyses in this study (Figure 1). 5 pairs and 397 ultrasonic vocalizations were analyzed in this study. These ultrasonic vocalizations were grouped according to individuals and behaviors. Data were tested for normality using the Univariate procedure (SAS, 2000). Because the durations were not a normal distribution, they were corrected through a Log10 transformation. The GLM procedure was used to compare the acoustic parameters. The effects of individuals, behaviors, and interaction between individuals and behaviors (individuals×behaviors) were tested on the durations and peak frequencies. Parameters are expressed as means±standard error.

RESULTS

There was difference in the duration and peak frequency: i.e. i) individuals (4 *df*; p<0.0001 and p<0.0001,

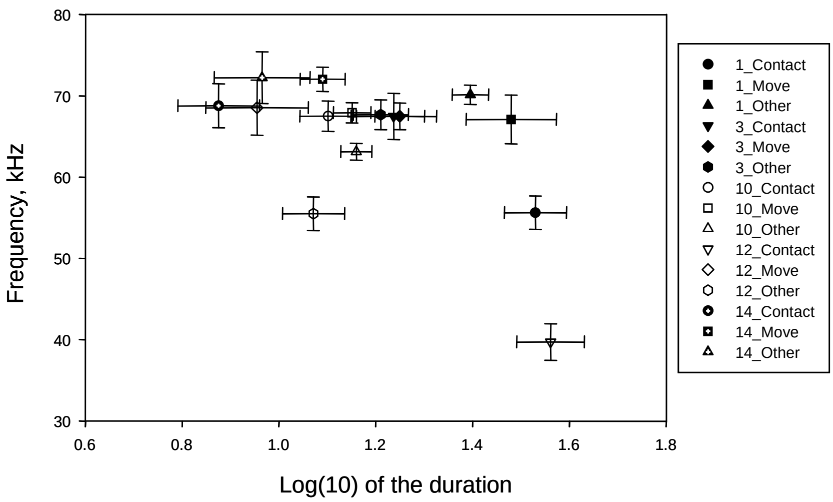

Figure 1. Effect of individuals and behaviors on acoustic features of ultrasonic vocalizations.

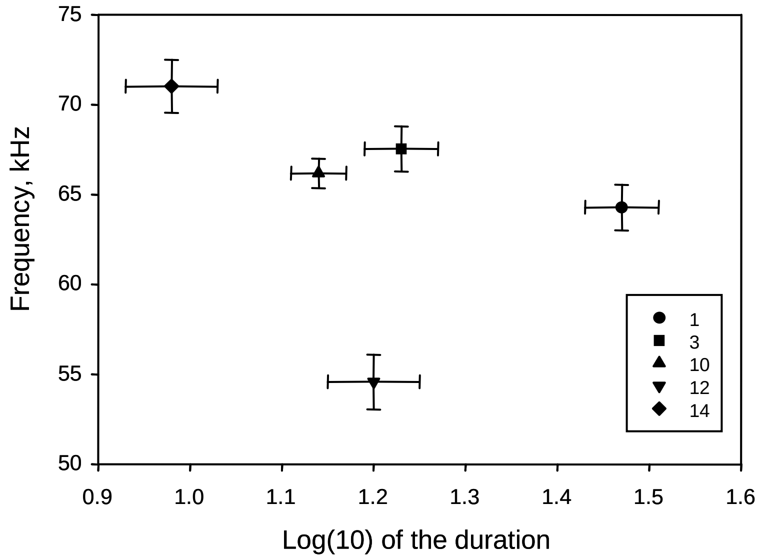

Figure 2. Effect of individuals on acoustic features of ultrasonic vocalizations.

respectively); ii) behaviors (2 *df*; p = 0.0667 and p<0.0001, respectively); iii) individuals×behaviors (8 *df*; p<0.0001 and p<0.0001, respectively).

Effects of individuals on acoustic features of USVs

We found that there were differences in the call duration and in the peak frequency among individuals (p<0.0001). Call duration of the 1st pair (35.9±0.04 ms; mean±SE) was the longest and that of the 14th pair (11.9±0.05 ms) was the shortest. Peak frequency of the 14th pair (71.0±1.48 kHz) was the highest and that of the 12th pair was the lowest (54.6±1.52 kHz; Figure 2). Especially, call durations of the 1st pair were the longest and call peak frequencies of the 14th pair were the highest throughout the whole behavior, except during bouts of 'contact' (Table 2; p<0.0001).

Table 2. The number of the ultrasonic vocalizations and average (±SE) call duration and frequency at peak amplitude. Call features are described separately for each of the behavior categories described in Table 1, and for each pair of rats observed

Behavior	Pair no.	No. of calls	Parameters			
			Duration (ms)		Peak Freq. (kHz)	
Contact	1	19	44.7±0.07	*I : <0.0001	55.6±3.47	I : <0.0001
	3	10	18.0±0.04	B : 0.0667	67.5±2.09	B : <0.0001
	10	23	15.7±0.06	I×B : <0.0001	67.5±1.52	I×B :< 0.0001
	12	16	47.5±0.09		39.7±2.45	
	14	11	9.2±0.08		68.8±2.37	
	Total		27.0±0.03		59.8±1.06	
Move	1	9	34.6±0.09		67.1±3.38	
	3	30	20.1±0.04		67.5±1.25	
	10	52	16.7±0.03		67.9±1.12	
	12	7	11.3±0.12		68.5±4.13	
	14	36	16.2±0.06		72.0±1.11	
	Total		19.8±0.03		68.6±1.04	
Other	1	56	28.4±.03		70.1±1.10	
	3	24	19.0±0.05		67.7±1.98	
	10	77	19.0±0.04		63.1±1.12	
	12	19	13.8±0.06		55.5±2.13	
	14	8	10.4±0.08		72.2±2.89	
	Total		18.2±.03		65.7±0.90	

* I: Individuals, B: Behaviors, I×B: Interaction between individuals and behaviors.

Effects of behaviors on acoustic features of USVs

Our findings showed that there was no difference in the call duration (p = 0.0667), but there was difference in the peak frequency among behaviors (p<0.0001). Call duration and peak frequency during bouts of 'contact' were longer (27.0±0.03 ms; mean±SE) and lower (59.8±1.06 kHz) than during bouts of 'move' and 'other' behaviors. On the other hand, call duration during 'other' behavior (18.2±0.03 ms) was the shortest and peak frequency during bouts of 'move' (68.6±1.04 kHz) was the highest compared to other behaviors (Table 2 and Figure 3; p<0.0001).

Effects of individuals×behaviors on acoustic features of USVs

This result showed that there was difference in the call duration and in the peak frequency among the individuals and behaviors (p<0.0001). Call duration and peak frequency were the longest and the lowest during 'contact' behavior. Call peak frequency of the 14th pair was the highest throughout the whole behavior and call duration of the 14th pair was the shortest during bouts of 'contact' (Table 2; p<0.0001). During 'move' behavior, peak frequencies of calls of entire pairs were near 70 kHz.

DISCUSSION

Sales (1979) reported that call duration tended to be shorter during isolation and call frequency tended to be longer in frequency than during handling calls. This was similar to data reported by Wöhr et al. (2008) in which 50 kHz calls were found in relatively high numbers during short isolation. In these studies, there was no difference in the call duration, but there was difference in the peak frequency among the behaviors. This observation was most likely due to no detection of the 20 kHz call in this study and this is comparable to that reported by Sales (1972) with short pulse. This result contributes further evidence that frequency of call is associated with duration of call.

Frequencies of ultrasonic vocalizations emitted by individual rats changed, ranging from 40 to 71 kHz, in this study. This result is consistent with earlier findings that call frequency of 35-70 kHz is known as a 50 kHz call (Sales, 1972; Blanchard et al., 1990; Kaltwasser, 1990; Brudzynski and Pniak, 2002; Burgdorf et al., 2007). According to Knutson et al. (1999) and Knutson et al. (2002), long 22 kHz ultrasonic vocalizations may indicate a state of negative activation, whereas short 50 kHz ultrasonic vocalizations may indicate a state of positive activation. However, we found that frequencies of ultrasonic vocalizations changed with a frequency ranging from 60 to 70 kHz which were emitted by behaviors and there was a difference in the peak frequency among the behaviors (p<0.0001). Thus, it is necessary to classify ultrasonic vocalizations into behaviors, although ultrasonic vocalizations in the 60-80 kHz range have been obtained in response to injection of antimicrobials (Dinh et al., 1999). Likewise, Fu and Brudzynski (1994) found that 50 kHz calls were recorded by injection of glutamate. We therefore suggest that high frequency ultrasonic vocalizations need to be subdivided and effects of individuals and behaviors must be considered to assess emotional state of rats.

This result is comparable to that of Brudzynski and Pniak (2002), in which 50 kHz calls were produced in anticipation of a social contact, although peak frequency of call during 'contact' behavior was lower than that of call during other behaviors in this study. This showed that call frequency is no different when a rat contacts a known cage-mate compared with an unknown cage-mate, so it seems

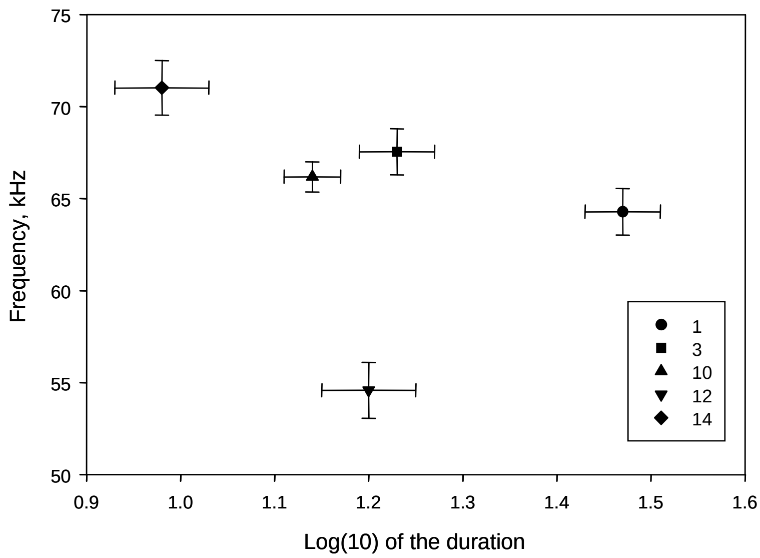

Figure 3. Effect of behaviors on acoustic features of ultrasonic vocalizations.

that cage-mate had no effect on the contact behavior-related ultrasonic vocalization.

In this experiment, 40 kHz calls were often found during bouts of ‘contact’ and ‘other’. 40 kHz ultrasonic vocalizations predominate during infancy (Noirot, 1968) and when pups are separated from their mother (Miczek et al., 1991). Brudzynski et al. (1993) reported that repeated hand touch applied to the neck of rats induced ultrasonic vocalizations, 2.6% of which were within 44-67 kHz. However, this result does not seem to be associated with previous studies. Rather, the 40 kHz calls in this study may be associated with behaviors because ‘contact’ and ‘other’ were not movement as shown in Table 1. High frequency ultrasonic vocalizations were also detected more on ‘move’ and ‘other’ behavior than during ‘contact’ in this study. This observation was similar to data reported by Knutson et al. (1998) who showed that high frequency ultrasonic vocalizations were linked to a motivational state rather than specific play behaviors or general activity.

Based on this experiment, it is not clear why the call frequency of the 12th pair was lower than that of others. However, it could merely be explained by individual difference because all of the environmental factors were controlled for all pairs. The variety of individuals makes it difficult to understand the ultrasonic vocalization. Hence, USVs as a robust indicator can be used to assess emotional states and welfare if we find the cause of the individual difference.

In conclusion, these results showed the spectrographic features of rat ultrasonic vocalizations among individuals and behaviors. Moreover, our findings show that acoustic features of ultrasonic vocalizations are influenced not only by individual but also by behavior. Our study indicates that effects of individual and behavior should be considered to assess emotional state using ultrasonic vocalizations.

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